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To:

European Research Office (9851 DU),
U.S. Department of the Army,

FRANKFURT am MAIN, Germany,

A.P.O. 757, U.S. Forces.

Subject of the Research:

Research on the synthesis of compounds containing an unsymmetrical triazinering. These studies may include compounds having unsymmetrical triazine rings with different structures; synthesis of compounds with substituents in the triazine or adjacent ring will be investigated.

Name of Contractor:

Prof. W. Berends,
Director Biochemical and Biophysical
Laboratory of the Technological
University,
Julianalaan 67,
Delft. (The Netherlands).

Contract number:

DA - 91 - 591 - EUC - 2099.

Type and number of report: Final Technical Report.

Period covered by the report: 1 January 1962 - 1 January 1963.

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Fatal food poisonings have repeatedly occurred amongst the natives of the densely populated parts of Mid-Java in Indonesia. These were caused by the eating of coconut-products ("bongkretek") that had been inoculated with moulds (*Rhizopus oryzae*). Mertens en van Veen¹⁾ were the first to make a study of the background of these poisonings round about 1930. An extensive investigation brought to light the fact that sometimes a bacterium developed instead of the mold with which the defatted coconut was inoculated and this secreted a very active poison. This "bongkretek bacterium" was identified as belonging to the genus *Pseudomonas* and it got the name "*Pseudomonas cocovenenans*".

The toxic compound, bongkretekic acid, was isolated from cultures of this microorganism on moist, defatted copra. The toxicity of bongkretekic acid is very general, for it acts not only on human beings but on all the animals tested (monkeys, rats, rabbits, pigeons) while, at the same time, the substance showed powerful antibiotic properties towards, many microorganisms (yeasts, bacteria, fungi). Though some work on the pharmacological action of bongkretekic acid was done in 1936 this must be considered as out of date and it should be repeated as soon as sufficient pure bongkretekic acid will be available again. Bongkretekic acid poisonings are characterized by a severe hyperglycaemia soon followed by hypoglycaemia. Some years ago we have found G. van Holt, pharmacologist at Hamburg, willing to carry out a few experiments on the action of bongkretekic acid on rats. The lethal dose appeared to be about 2 mg per 100 g rat. After oral administration the animals showed an almost complete elimination of glycogen in the liver, that was accompanied by strong hypoglycaemia. Administration of glucose to 100 mg% did not prevent death of the rat, so that the hypoglycaemia itself is apparently not responsible for the lethal effect. The cumulative effect of bongkretekic acid and the sudden onset of a shock-condition are noteworthy. No definite damage to the Langerhans-islets could be established.

Bongkretekic acid is a highly unsaturated fatty acid. As a

free acid it is very labile and crystalline material has never been obtained as yet. The isolation and purification of bongkrekkic acid was taken up in this laboratory a few years ago. After having done a lot of work on the elucidation of bongkrekkic acid this was laid down in a thesis. The structure could be partially elucidated but much had still to be done. We had no intention to continue this work on the structure of bongkrekkic acid for some laboratories in England wanted to do this. For this I put the strain of *Pseudomonas cocovenenans* available. Half a year ago I was informed that no progress had been made, so we have taken up the research on bongkrekkic acid again.

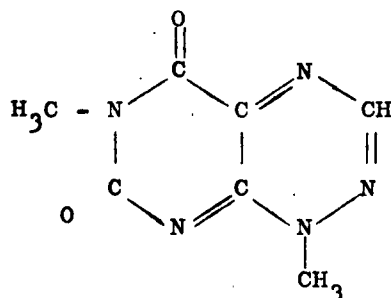
Our preliminary work on the structure of bongkrekkic acid was published in 1957 (D.H. Nugteren and W. Berends, Rec. 76 (1957) 13), whereas investigations on the mode of action of this compound were reported a few years later (W. Welling, J.A. Cohen and W. Berends, Biochem.Pharmacol. 3 (1960) 122).

Cultures of *Pseudomonas cocovenenans* are yellow. This colouring matter was also isolated by van Veen c.s. already before the second world war. It was called toxoflavin. Toxoflavin is pharmacologically highly active and it also shows strong antibiotic properties. Though a rather extensive account of the investigations leading to a structural formula were given the conclusions from this work seemed unacceptable to us and therefore a new systematic study was started. After having improved the cultivation and isolation of toxoflavin the structure was elucidated (P.A. van Damme, Miss A.G. Johannes, H.C. Cox and W. Berends, Rec. 79 (1960) 255),

After having finished our work on the structure of toxoflavin we initially hesitated between publishing our results or starting research on the synthesis. Finally it was decided to publish the molecular structure so as to give other scientists, probably being better organic chemists, a fair chance to synthesise toxoflavin. Even before having selected a team the synthesis was published by workers at the Midwest Research Institute (G. Doyle Davis, R.K. Robins and C.C. Cheng, J.Am.Chem.Soc. 83 (1961) 3904 and 84 (1962) 1724). These authors also announced their intention to synthesise several analogues of toxoflavin.

This work would have prevented us from doing anymore on this subject but support of the U.S. Army stimulated us to investigate the field of unsymmetrical triazines. Two rings occur in the molecule of toxoflavin, one of these is a partially unsaturated, unsymmetrical triazine. The unsymmetrical triazines belong to a rather unexplored field of organic chemistry, so it might be interesting to do investigations on structure-activity relations. At the same time many organic chemical problems might be expected. After preliminary work to get some experience with these compounds a study of the hydrogenation of unsymmetrical triazines is worthwhile. Experimental work on the hydrogenation of pyrazines has been going on in this laboratory for several years and the results, most of them still unpublished, are unexpected and they may lead to new ideas about the role of such compounds in certain biochemical reactions. It should be emphasized that no sharp outline of the program can be given at the outset for it will take some time before we are more or less familiar with the behaviour of compounds like these and how to synthesise them.

The structure of toxoflavin is:



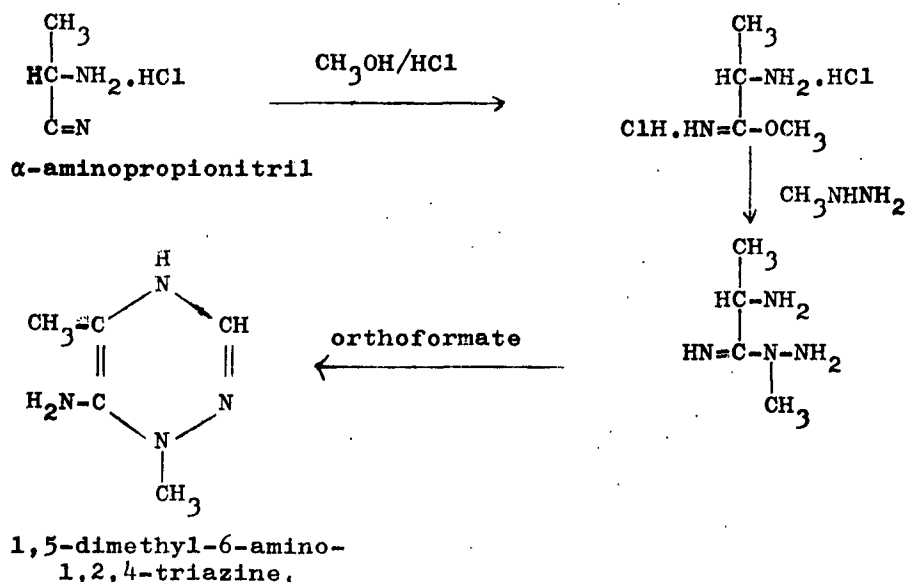
1,6-dimethyl-5,7-dioxo-1,5,6,7-tetrahydropyrimido-(5,4-e)-1,2,4-triazine.

The work that has been done in the last year concerns the following syntheses:

1. The synthesis of an unsymmetrical triazine closely resembling the one in toxoflavin.

- II. The synthesis of a pyrimido-as-triazine, in which the position of the rings in relation to toxoflavin is changed.
- III. The synthesis of a pyrazolo-pyrimidine.
- IV. The synthesis of a pyridino-as-triazine.

ad. I. Unsymmetrical triazines substituted by an amino group at position 6 are unknown in the literature. As seen from the molecular formula of toxoflavin an unsymmetrical triazine with such a substitution occurs in toxoflavin. We have been trying to synthesize 1,5-dimethyl-6-amino-1,2,4-triazine by the following methods.



Following the steps indicated in this scheme we soon got into trouble. In the first place from the work of Baksheev and Gavrilov (J.Gen.Chem.(U.S.S.R.) 22 (1961) 2077) it was already expected that the α -aminopropionimino-methylether-hydrochloride is a very unstable compound for they found an easy decomposition into an amide and an alkylchloride.

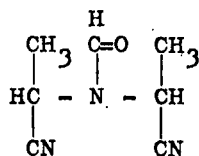
The next step is a reaction between an iminoether and methylhydrazine. According to a paper by Mengelberg (M. Mengelberg, Ber. 89 (1956) 1185) the reaction between an iminoether that bears an aminogroup at the α -position and ammonia leads to

coloured products that could not be purified to crystalline material. For carrying out this reaction the α -aminogroup has to be protected by acylation. Though Mengelberg used p-toluene-sulphonyl- and the carbobenzoxygroup we first wanted to try out the synthesis with an acetyl- and a formylgroup.

If the starting material is α -acetamido-propionitrile the final reaction product expected would be 6-amino-1,3,5-trimethyl-1,4-dihydro-1,2,4-triazine. Formylation of the aminogroup instead of acetylation would lead to 6-amino-1,5-dimethyl-1,4-dihydro-1,2,4-triazine.

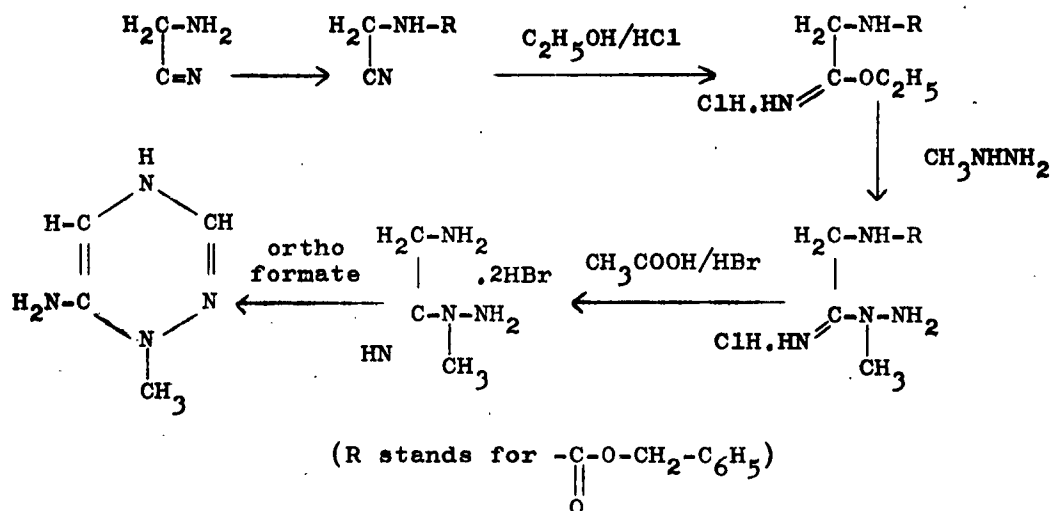
The acetylation of the nitril was a smooth reaction but when trying to convert this into an iminoether we ran into difficulties. No crystalline material could be isolated. Treatment of the oily product with methylhydrazine or ammonia gave no results either. The iminoether appeared to be highly hygroscopic. Hydrolysis took place very easily.

It is remarkably that formylation of the nitrile, in contrast to acetylation, did not succeed. Dark coloured oils were obtained from which a crystalline compound could be isolated. It could be demonstrated to have the structure.



In using the mixed anhydride of acetic and formic acid the reaction product appeared to be the acetylated compound. From all these time consuming experiments, especially for the attempts to bring the reactionproducts to crystallization, it became clear that another acylgroup had to be introduced for the protection of the aminogroup. We first preferred to try out the carbobenzoxy-group, for this one is easily to remove with a hydrobromic acid solution in glacial acetic acid or by catalytic hydrogenation.

The following scheme has been tried.

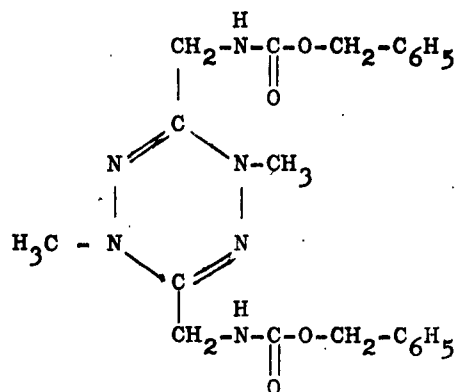


And the same reaction scheme with α -aminopropionitrile, giving 6-amino-1,5-dimethyl-1,4-dihydro-1,2,4-triazine.

We observed that the influence of moisture on carbobenzoxyminoethylether.hydrochloride is less than it is in synthesising iminoether from acetylated α -aminopropionitrile. Though it influences the yield of iminoether it does not give a complete failure.

We were able to synthesise carbobenzoxymino acetonitril, carbobenzoxyminoacetiminioethylether.hydrochloride, carbobenzoxyminoacetamidrazon.hydrochloride and aminoacetamidrazon.dihydrobromide.

The synthesis of iminoether was performed according to Mengelberg. The reaction between iminoether and methylhydrazine gave some complications. A solution of the iminoether in absolute alcohol and methylhydrazine in 20% excess at room-temperature gave white crystalline solid in a short time; this turned out to be ammoniumchloride. After about 24 hrs. a yellow crystalline product separated, that on analysis gave a ratioformula $\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}_2$, while the formula for the amidrazon had to be $\text{C}_{11}\text{H}_{16}\text{N}_4\text{O}_2$. Ammonia had disappeared as ammoniumchloride. Determination of the molecular weight according to the method of Signer: $M=446$ and $M=414$. As a dimer, $(\text{C}_{22}\text{H}_{26}\text{N}_6\text{O}_4)$, had to have $M=438$ we supposed a tetrazine of the following structure might be the reactionproduct:



That it is very well possible to synthesize tetrazines this way was demonstrated by Wiley and coworkers (R.H. Wiley, C.H. Jarboe, F.N. Hayes, J.Org.Chem. 22 (1957) 835) who synthesised several tetrazines.

Tetrazines may be formed from iminoethers and hydrazine. The main reaction product is, according to the literature, dependent on the temperature and the amount of hydrazine.

Condensation of carbobenzoxyamino acetiminoethylether-hydrochloride with methylhydrazine at -10° C, however, gave carbobenzoxyaminoacetamidrazon hydrochloride.

From this product the carbobenzoxygroup was removed and aminoacetamidrazon.dihydrobromide could be obtained.

The ringclosure has not yet given satisfactory results. Using orthoformate we obtained a dark brown liquid from which we could not isolate a crystalline compound. In using acetic anhydride in pyridine a crystalline product appeared to be pyridine-hydrobromide. The ringclosure will be tried again starting from the free amidrazon.

The reactionproduct of the carbobenzoxylation of α -amino-propionitrile appeared to be α -carbobenzoxyamino-propionamide. If, however, we used pyridine instead of a sodiumhydroxide solution we obtained α -carbobenzoxyaminopropionitrile.

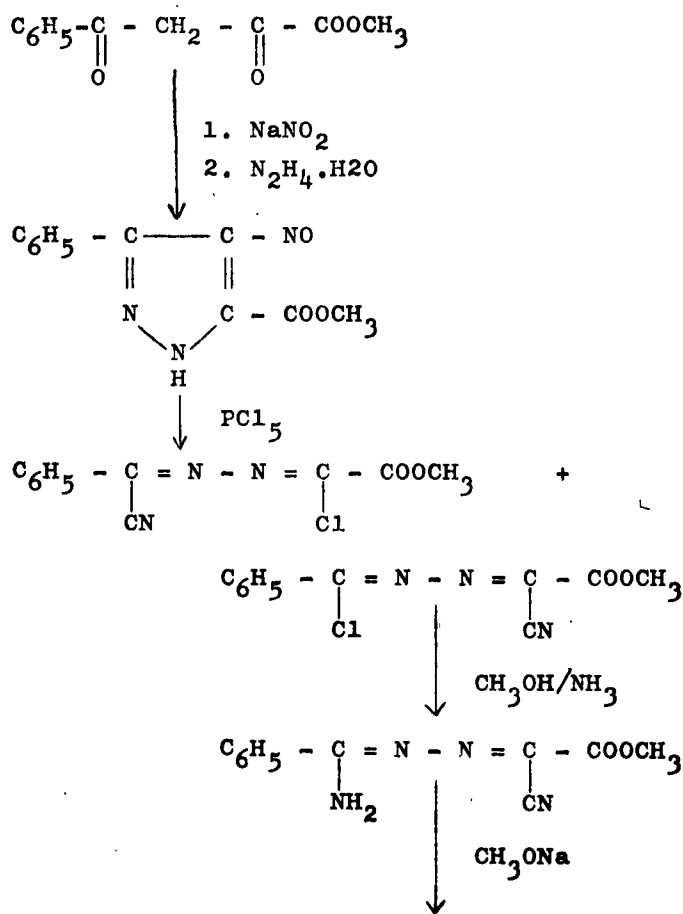
The preparation of iminoether caused difficulties on account of failure to crystallize and sensitivity to moisture. We took precautions by adding absolute alcohol and ether from closed apparatus, directly in the reaction vessel, and increased the reactiontime from several hours to 48 hrs. We observed that after

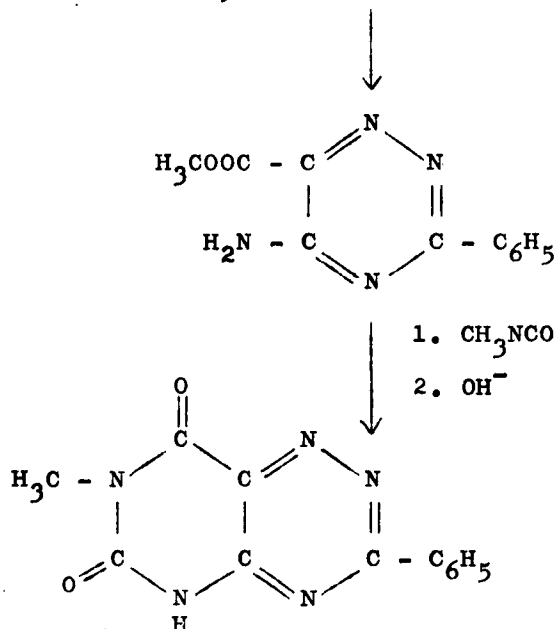
24 hrs. an oil appeared, which solidifies after 48 hrs. After that we removed excess ether, alcohol and hydrogenchloride in vacuum over phosphorous pentoxide and solid sodium hydroxide.

Though we have not yet worked up the product, we expect that we have been successfull at last in synthesising α -carbobenzo-cyaminopropioniminoethylether-hydrochloride.

ad. II. In our studies of literature we found a compound synthesised by Fusco and Rossi (R. Fusco and S. Rossi, Tetrahedron 3 (1958) 209), namely 5-amino-6-carbomethoxy-3-phenyl-1,2,4-triazine. This compound might be used for the synthesis of an interesting derivative.

The synthesis of this derivative is as follows:





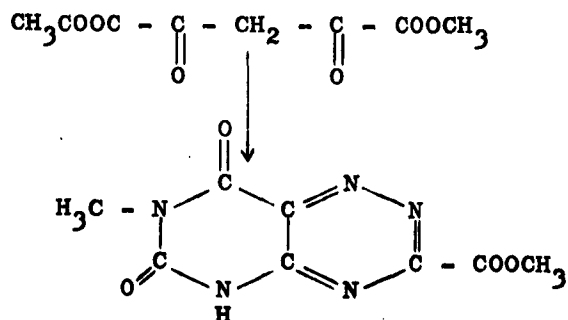
3-phenyl-7-methyl-6,8-dioxo-5,6,7,8-tetrahydro-pyrimido-(5,6-e)-1,2,4-triazine.

The separation of the two diazabutadiene derivatives appears to be a difficult problem.

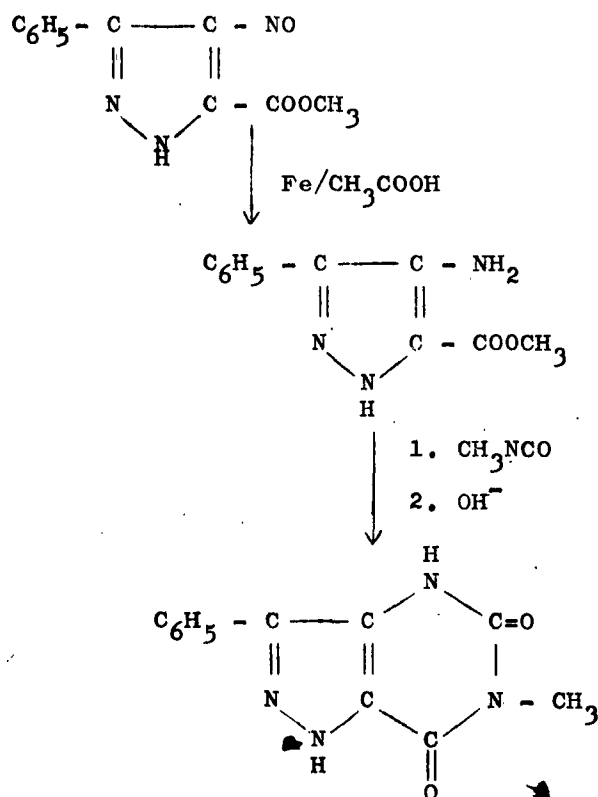
When we tried to close the second ring with methylisocyanate, the sodium salt of a carboxy-hydroxy-phenyl-1,2,4-triazine was obtained. Whether it is 6-carboxy-3-phenyl or 3-carboxy-6-phenyl is not known as yet.

If this line of the work may appear more complicated than expected it will not be continued.

It would be much easier, to start from a symmetrical compound for no problem of a separation would be involved. Starting from oxalylpyruvicmethylester and following the same route as Fusco and Rossi, a pyrimido-as-triazine should be the endproduct; as is illustrated:



ad. III. In trying to synthesise the pyrimido-as-triazine the intermediate 3-phenyl-4-nitroso-5-carboxymethoxy-pyrazole was obtained. This compound was easily reduced to the amino derivative. By a reaction with methylisocyanate a second ring could be closed:



We have succeeded in synthesising this compound. It has been forwarded for pharmacological testing.

ad. IV. According to Wolf and coworkers (F.J. Wolf, K. Pfister, R.M. Wilson and C.A. Robinson, J.Am.Chem.Soc. 76 (1954) 3551), who synthesised a 1,2,4-benzotriazine, we will try the following reaction sequence:

